CONTROLS ON BUD ACTIVATION AND TILLER INITIATION IN TALLGRASS PRAIRIE: THE EFFECT OF LIGHT AND NITROGEN

By

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CHAPTER I

Effects of Nitrogen and Light Availability on Bud Banks

ABSTRACT

Perennial grass populations in tallgrass prairie ecosystems are maintained primarily through vegetative outgrowth from their belowground bud banks. Resources in these ecosystems that have been found to limit plant productivity include nitrogen and light. I tested the Tomlinson and O'Connor (2004) theory, on the interactions of two environmental cues, nitrogen and light quantity, and the roles they have on bud outgrowth and tiller initiation in six perennial grass species in two functional groups (C_3 and C_4 photosynthetic pathways). I hypothesized that (1) an interaction between nitrogen and light availability regulated bud activation and tiller emergence in the two functional groups (C_3 species and C_4 species), (2) nitrogen would have the largest effect on the activation of buds, and (3) responses to the two environmental cues would differ between the two functional groups. Environmental growth chambers were used to conduct all studies, plants received one of three NH_4NO_3 amendments or water (control), and were placed in a light treatment chamber or a dark (control) chamber. Strong interactions between nitrogen and light influenced bud outgrowth in the C_3 species, but not in the C_4 species. When I assessed the impacts of nitrogen as a key cue in tiller

initiation in all six species, C_3 species responded favorably to N, while C_4 species did not. These results indicate that another abiotic environmental cue may be influencing the C_4 species. The results of this study suggest that environmental cues such as these that impact belowground bud bank dynamics in the tallgrass prairie have to potential to significantly impact grassland dynamics in response to current and future global changes.

INTRODUCTION

Most rangelands worldwide are dominated by perennial grasses, many of which are maintained largely through the maintenance of their belowground population of dormant meristems or "bud banks" (Harper 1977). Bud banks have recently been recognized for the key role they play in the recruitment of grass populations in tallgrass prairie ecosystems and have been found to be of much greater importance in plant population persistence and dynamics than seed rain or seed banks (Glenn-Lewin et al. 1990, Pyke 1990, Hartnett & Keeler 1995, Hartnett & Fay 1998, Benson et al. 2004, Benson & Harnett 2006). Studies have also demonstrated the key role of belowground meristem limitation in influencing patterns of grassland productivity, and have showed how the effects of prescribed fire regimes, grazing, or other factors can be mediated through their influence on belowground bud populations (Dalgleish and Hartnett 2006, Dalgleish and Hartnett 2009). Because belowground bud banks impact various aspects of grassland ecosystem dynamics, a sound understanding of the proximal abiotic environmental cues that regulate bud development, dormancy, and outgrowth is essential to accurately predict rangeland responses to changing abiotic environmental conditions (e.g. regional N enrichment and alterations in precipitation and temperature or shifts in limiting resources) (Seastedt and Knapp 1993).

In tallgrass prairie ecosystems, nitrogen, water, and light are three resources that regulate plant productivity. The relative importance of these three limiting resources varies spatially and temporally, with nitrogen often being the most limiting resource in these systems (Owensby et al. 1970, Seastedt and Knapp 1993, Knapp et al. 1998). Consequently, population dynamics of prairie plants, as well as vegetation growth and production are expected to be influenced by these limitations. Previous research conducted in the tallgrass prairie has indicated increased water availability to be positively correlated with increased bud bank densities (Dalgleish and Hartnett 2006) and nitrogen addition has positive effects on bud outgrowth and tiller emergence and genet growth rates in some grassland species (Dalgleish et al. 2008). Light plays a key role in many aspects of plant development. Elongation of stems, increase in leaf area and increase in aboveground tillers have been documented for plants exposed to increases in light quantity and light spectral quality, in particular, the red to far red (R:FR) ratio (Tucker 1975, Smith 1982, Deregibus et al. 1983, Ballare et al. 1994). Because variation in aboveground net primary production (ANPP) in tallgrass prairie ecosystems can be attributed primarily to differences in tiller density, rather than tiller size (Hartnett and Fay 1998), these essential resources may be driving patterns of bud dormancy, outgrowth, and tillering.

Based on a synthesis of previous studies conducted on the mechanisms that stimulate the outgrowth of dormant buds in grasses, Tomlinson & O'Connor (2004) proposed an integrated theory. Their aim was to link key mechanisms responsible for lateral bud outgrowth. The key mechanisms in their model include apical dominance, photosensitivity to the red to far red (R:FR) ratio, and resource (nitrogen) availability

(Briske and Derner 1998). Tomlinson and O'Connor's integrated model describes the role that each cue plays in controlling and regulating bud dormancy and tiller recruitment from bud banks (Fig. 1). Of these three cues, Tomlinson & O'Connor (2004) predict nitrogen to be the strongest promoter of dormant bud outgrowth and tiller emergence in grasses, and will therefore act as a key environmental factor regulating bud dynamics.

The key objective of the study was to test the Tomlinson and O'Connor (2004) model predictions for perennial grasses in the tallgrass prairie. In this chapter, I specifically focus on the interactions of available nitrogen and the light environment on regulating bud dormancy and outgrowth. This research will assess general applicability of this model by examining the factors controlling bud dormancy and activity in several dominant rhizomatous and caespitose grass species from a mesic North American rangeland. I tested three hypotheses based on Tomlinson and O'Connor's (2004) model predictions:

$H_{1:}$ Nitrogen and light interact to influence bud outgrowth in both C_3 and C_4 species of grasses.

H_2: Nitrogen plays a key role in the activation of buds and initiating tillering.

H_3: The responses to the two environmental cues will differ between the two functional groups (C_3 vs. C_4 species).

In testing these hypotheses, I assessed interactions between nitrogen availability and light as important cues. The results will additionally provide information on whether nitrogen serves as a qualitative cue (e.g. non-dose dependent developmental trigger) necessary to trigger a tillering response or if nitrogen addition has quantitative (dose-dependent) effects, determining the plants' nutritional status. This research is the first

study to empirically test the model predictions that Tomlinson and O'Connor (2004) proposed. It explores unanswered questions to further our knowledge on the roles of bud banks in regulating grassland ecosystem dynamics and plant physiological processes. The outcome of this study will improve our understanding of the mechanisms that are driving patterns in productivity and increase our ability to predict grassland responses to environmental change phenomena such as nitrogen enrichment.

MATERIALS AND METHODS

Collection Site:

All plant materials were collected from Konza Prairie Biological Station (KPBS). This station is a 3467-ha tallgrass prairie research site, located 10 km south of Manhattan, KS, USA within the Flint Hills region of northeastern Kansas (39°05' N, 96°35' W). The site is owned by the Nature Conservancy and managed by Kansas State University, Division of Biology. KPBS is dominated by perennial, warm-season tallgrasses such as big bluestem (*Andropogon gerardii),* little bluestem (*Schizachyrium scoparium),* and switchgrass (*Panicum virgatum),* but contains a diverse mixture of cool-season graminoids, legumes, and other interstitial forb species, as well as shrubs (Towne 2002, Freeman 1998). KPBS is divided into 64 replicate watershed units, each containing distinct topographical gradients, including upland, slope, and lowland areas. Fire and grazing regimes are also assigned to each watershed. Fire regimes include 1-, 2-, 4-, 10-, and 20-year frequencies and grazing regimes include grazed or ungrazed by native herbioves (e.g. bison) or cattle. All of my samples were collected from annually burned, ungrazed watersheds.

Selection of Species:

Photosynthetic pathways of C_3 and C_4species play a critical role in flowering phenology, growth period, drought tolerance, and ultimately geographic distribution. C_4 or warm-season grass species grow throughout the summer months, with flowering typically occurring in the late summer months. During this period of new tiller growth and maturation, new buds are forming, joining older buds, to form the overwintering dormant bud bank, some of which will subsequently tiller the following year during the growing season. Alternatively, C_3 or cool-season grass species have a growing season that begins in early spring, with flowing occurring from April to June. Production of dormant buds occurs during this period as well, and continues throughout their growing season. Studies conducted on the C_3 species *D. oligosanthes,* and the C_4 species *A. gerardii*, showed that while both species became dormant over the winter season, *A. gerardii* used buds to overwinter, while *D. oligosanthes* used juvenile tillers to survive the winter dormancy. A second difference in the bud bank dynamics between the two groups is that *D. oligosanthes* has an additional dormancy period in the summer due to high temperatures, where it uses buds to survive the dormancy (Ott 2009).

The belowground perennating organs (e.g. crowns and rhizomes) of established C_4 grasses (*Andropogon gerardii*, *Schizachyrium scoparium* and *Panicum virgatum*) with their dormant buds and C_3 grasses (*Koeleria macrantha, Elymus canadensis*, and *Dichanthelium oligosanthes*) with their dormant buds were collected on watersheds N1A, K1B, and 1D on KPBS. C_4 materials were collected in February, when their buds were dormant, while the C_3 rhizomes were collected in July, corresponding to their summer bud dormancy (Ott 2009).

Belowground Sampling:

Plant materials were harvested to a depth of 10 cm and all interconnected and belowground structures (buds, rhizomes, roots, etc) were stored in large plastic containers and transported to Oklahoma State University. The samples were washed free of soil, sorted by species, and roots trimmed to 3 cm. Rhizomes were then placed into flats of moistened Medium A-2 grade vermiculite and were refrigerated at 2-4°C in complete darkness until experimental set-up.

Experiment Set-up:

Experiments were conducted in Controlled Environmental Chambers, (Conviron-PGW 36, interior dimension: 98"Wx54"Dx93"H, growth area: 36ft^2, and growth capacity: 240 ft^2), housed within the Controlled Environmental Research Lab (CERL) on the campus of Oklahoma State University in Stillwater, OK. Replicate studies were conducted on all six species (C_4 and C_3) of grasses in the spring and fall of 2009. Perennating organs of similar size of each individual species were transplanted into square plastic pots (9 cm length x 8 cm depth) containing vermiculite. Each perennating organ was placed within 1 cm of the vermiculite surface and randomly arranged in growth chambers. Because we observed green tillers during the dormant season collection on both *D. oligosanthes* and *K. macrantha*, prior to placing these species into pots, live tillers were counted and recorded.

To assess the effects of bud outgrowth under photoperiods and temperature regimes that are similar to actual conditions in the tallgrass prairie during the growing season for each functional group, chambers were set under a photoperiod of 14 hours at 30/20°C day/night temperature for the C_4 (warm-season) species and 20/12°C day/night

temperatures for the C_3 (cool-season) species. A full dark treatment was included as a control. Each species had six replicates subjected to each of the nitrogen treatments, for a total of 24 pots/species in the light treatment and 24 pots/species in the dark control treatment.

Plants were monitored and irrigated every 2-3 day, and the number of buds released from dormancy and emerging as juvenile tillers were counted and recorded daily for 3 weeks or until no new tiller initiations were observed. The belowground plant components were then washed free of vermiculite and number of live buds and live tillers (>2cm) present were counted using a dissecting microscope. The number of live residual buds + the number of live newly emerging tillers provides the starting number of buds on the belowground structures at experimental set up.

Nitrogen and Light Availability:

At experimental set-up and 2 days later, pots were supplemented with 50 mL of one of three supplemental nitrogen concentrations: 50, 150, 350 mg NH_4NO_3/L N (low, medium, and high), following the methods of McIntyre & Cessna (1991). The remaining six pots served as a control and received equivalent amounts of water (50 mL). Two light conditions were conducted simultaneously in separate growth chambers; (1) pots subjected to dark conditions (24-hr) and (2) pots subjected to daylight conditions (14-hr) with light intensity at 960 micromoles/m^2/s, lamps-balanced spectrum using florescent and incandescent light bulbs.

Statistical analysis:

The following equation was used to determine the proportion of buds that broke dormancy and grew out into juvenile tillers in each of the treatments:

$$\frac{T_e}{T_e+(B_d-T_i)} = \text{proportion of buds initiating into tillers}$$

where T_e=number of emerging tillers (>2cm) and B_d=number of remaining dormant (inactive) buds recorded at the end of the experiment for each replicate, and T_i = number of juvenile tillers present at the beginning of the experiment. Proportional data of C_4 species were logit transformed prior to analysis to reduce heterogeneity of variances. Untransformed data are presented on figures. For each species, the effects of N and light treatments on proportion of bud producing tillers were analyzed via two-way ANOVA using SAS version 9.2 software (SAS Institute Inc, Cary, NC, USA), and the significant levels of differences are reported for $P \leq 0.05$.

RESULTS

Response of C_3 grasses to nitrogen amendments and light availability:

All three C_3 plant species responded similarly to nitrogen amendments when maintained under 14 day photoperiods of light availability. The low nitrogen amendment (50 ppm N per pot) resulted in a stimulation in bud outgrowth and tiller initiation, compared to the amended control (Figure 1-2, A –C). However, this stimulation in tiller initiation was not observed in rhizomes that were amended with my medium or high nitrogen concentrations (150 ppm or 350 ppm per pot). I observed a strong interaction between nitrogen amendment and light availability ($P<0.05$ for each C_3 species). The significant stimulation of bud outgrowth at low nitrogen in the light was not observed in total dark. In light, the peak in tiller initiation occurred at low nitrogen concentrations, however in the dark, the peak stimulation of tiller emergence occurred at the highest nitrogen concentrations in two of the three species. There was no effect of nitrogen in *E. canadensis* maintained in the dark (Figure 1-2, A-C).

Response of C_4 grasses to nitrogen amendments and light availability:

I did not observe a clear or consistent response to nitrogen amendments with the three C_4 grasses observed in this study. Nitrogen had no effect on tiller initiation under either light or dark conditions (Figure 1-3, A-C), and there was no interaction between light and dark treatments ($P > 0.05$) for any of these three species. There was a trend of decreased meristem initiation under the low nitrogen amendment in *P. virgatum*, although this response was not as pronounced as the increase observed under this fertilization level for the C_3 species (Figure 1-2, A-C). This decrease was also not consistent across light treatments, as the decrease was observed in *P. virgatum* under dark conditions and with *A. gerardii* when maintained under a 14 hour photoperiod (Figure 1-3 A-C).

DISCUSSION

The results of this study clearly indicate that C_3 and C_4 tallgrass prairie species differ strongly in the factors that regulate bud dormancy and juvenile tiller initiation. Strong interactions between light and nitrogen were observed in the C_3 species supporting the first hypothesis, but this hypothesis was clearly not supported by the C_4 species. Further exploration into why these results were observed will be discussed in more detail below.

In my second hypothesis, I proposed that nitrogen plays a primary role in the activation of buds into tillers. In the C_3 grass species, I found this to be the case. The significant boosts in tiller initiation at low nitrogen levels were found to be greater than three-fold, compared to control treatments in *D. oligosanthes* and nearly two-fold, compared to control treatments in the other two C_3 species. Therefore, this hypothesis

was supported as well. However, this was not observed across both light and dark treatments. In C_3 species, light significantly altered the proportion of buds that initiated into tillers, indicating these two cues interact to maximally stimulate tiller initiation in all three of these species. In the C_4 grass species, the second hypothesis is rejected. Nitrogen does not appear to play a key role in bud activation and tiller initiation in these species. When we assessed the effects of nitrogen and light separately, I observed a consistent pattern among treatments, with very little variation between these resources.

Our third hypothesis can be accepted unconditionally. The evidence for differences in responses to light and nitrogen between the two functional groups have been clearly observed in the acceptance and rejection of both of our first and second hypotheses. The differences between the two physiological groups are apparent, in both their response to nitrogen as well as light treatments. All three C_3 species had a similar response to low nitrogen treatments in the light, while I observed a different response in C_4 plant species. C_4 plant species response to nitrogen and light was not as pronounced, the response to the nitrogen treatments were similar in both light treatments (light and dark) and I saw a slight increase in bud outgrowth in the high nitrogen treatments in both light and dark conditions.

These results of my first hypothesis indicate that light and low levels of nitrogen appear to maximize bud outgrowth and tiller initiation in C_3 species, while there seems to be another environmental trigger influencing tiller initiation in C_4 species. A previous study conducted by Ott (2009), found that in the C_4 species, *A. gerardii*, active buds were recruited from the dormant bud bank in late March and tillered in late April. These data correspond to an average April temperature on KPBS of 21°C during the daytime and

9°C at night. This is 9° and 11° lower respectively, than the 30/20°C temperature settings for C_4 species in my study. Ott (2009) also reported that *D. oligosanthes* tillered during the months of September and October, when temperatures averaged 24°C during the day and 9°C at night, only 4° higher and 4° lower respectively than the 20/12°C temperature settings for this study. Therefore, the growth chamber settings of the C_3 species in my study more closely resemble the optimal temperature requirements for tillering in the field, than the settings of my C_4 species. Therefore, it's plausible that temperature and possibly photoperiod could play a key role in the activation and tiller initiation of C_4 species rather than nitrogen and light alone.

Tomlinson and O'Connor's (2004) model predicted that nitrogen would be the strongest promoter of dormant bud outgrowth and tiller emergence in grasses is supported in the C_3 species. So an important question is whether nitrogen is a qualitative cue, directly involved with stimulating a tillering response (non dose-dependent) or if nitrogen addition has quantitative (dose-dependent) effects that determine the plants nutritional status? For the C_3 species, nitrogen seems to be directly involved in the stimulation of bud development at optimal levels, indicating it is not a quantitative, dose-dependent response. The C_4 species appear to be triggered by another important environmental cue, possibly temperature, as discussed above.

That we observe C_3 species following the model predictions constructed by Tomlinson and O'Connor is not surprising. All of the species included by their prediction model were C_3 species. Because the differences in physiology between the two functional groups have been well documented, it's reasonable to assume that differences in their responses to environmental cues would also exist. Based on my results, factors that

promote nitrogen enrichment into the grassland system, paired with increases in light availability (e.g. fire, land use changes) could potentially cause a shift in species composition from C_4 dominated grasslands to C_3 dominated grasslands. C_4 species have superior strategies to cope with warmer temperatures and decreased precipitation, but in the context of bud outgrowth, little work has been done. C_4 species did not follow the Tomlinson and O'Connor (2004) model, indicating that the next logical step will be to create a separate integrated model naming factors influencing bud outgrowth and tiller initiation for the C_4 species.

Other factors that Tomlinson and O'Connor (2004) predict as playing a key role, but were not directly addressed in our study was the role of hormones and light spectral quality on bud outgrowth and tiller initiation. The two hormones named as playing a key role were auxins and cytokinins. The hormone auxin has been found to regulate apical dominance and inhibit lateral (axillary) bud outgrowth. The second group of hormones, cytokinins, promotes cell division, lateral bud outgrowth, and the mobilization of plant nutrients and may therefore be responsible for the stimulation of bud outgrowth (Taiz and Zeiger 2006). Light spectral quality was the other cue named by Tomlinson and O'Connor (2004) to be interacting with the hormones and nitrogen, to affect bud outgrowth and tiller initiation in grasses. Recent studies suggest that phytochrome sensitivity to R:FR may actually manipulate auxin rates, resulting in an inhibition of bud outgrowth (Cline 1994, Kraepiel and Miginiac 1997, Tomlinson and O'Connor 2004). This study compares the response to the simple presence or absence of light, but a sensitivity to light quantity or quality could be an alternative cue that stimulates C_4 bud

activation. The difference in sensitivities to light spectral quality will be the focus of the next chapter.

Ott (2009) found that *A. gerardii* dormant buds grew out to juvenile tillers more frequently on adult flowering tillers than on vegetative tillers. She suggested that environmental cues, such as temperature and photoperiod may influence a size threshold that may eliminate this discrepancy. This study offers a possible alternate cue that triggers bud initiation in C_4 grasses, and that is subsequent plant development. A sensitivity to light was observed in the C_3 species studied, therefore, further exploration of light (quantity and quality) as a cue, would allow us to further test the Tomlinson and O'Connor model and possibly provide us with the answers as to what is acting as a trigger in the C_4 grass species.

It's important to recognize that when determining the potential long-term impacts of environmental cues such as increasing nitrogen, or light quantity on belowground bud banks, interactions of these complex systems will have a significant effect. Multiple limiting resources are interacting together to create this complex ecosystem (Knapp et al. 1998b). As we continue to search for the answers to how these systems will respond to global change, we must take a closer look at the interactions of many different environmental cues. Increases in human populations, fossil fuel consumption, and intensification of agriculture continue to occur globally, with a concomitant acceleration of N deposition. (Vitousek et al. 1997, Van Drecht et al. 2005, Holland et al. 2005). The results of this study indicate that bud outgrowth in C_3 species is sensitive to an interaction between nitrogen and light availability. This indicates that factors that affect these two resources could potentially affect grasslands by shifting species composition from a C_4

dominated grassland to a C_3 dominated grassland. Therefore, differences in meristem response to these environmental alterations may be critical in determining patterns of species composition and population dynamics in response to current and future global changes.

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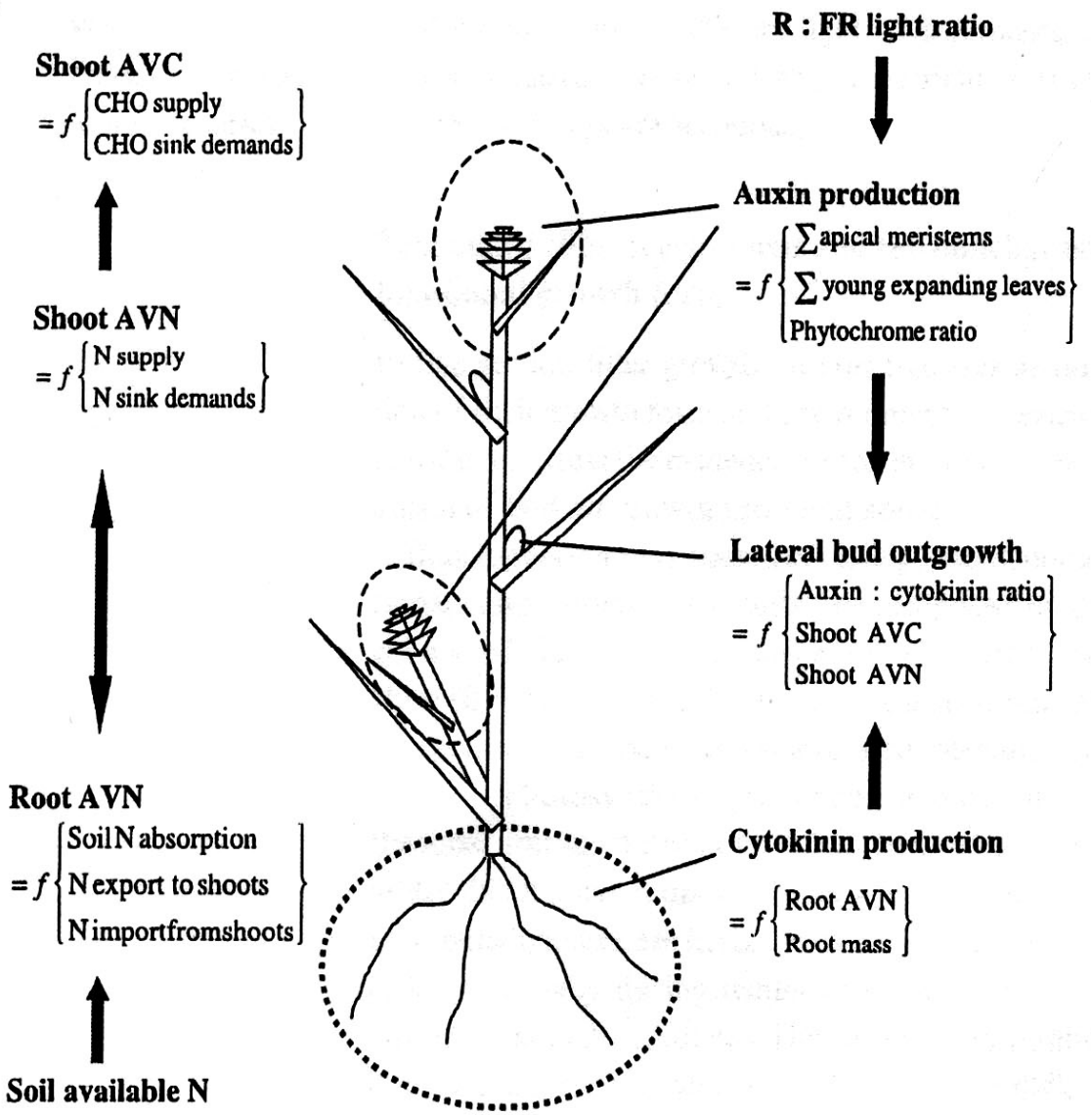

Figure 1-1. Tomlinson and O'Connor (2004) integrated model of the processes controlling lateral bud outgrowth. All variables are shown as functions (*f*) of the variables that drive them. (R:FR = red : far red light ratio; CHO = carbohydrate; N = nitrogen; AVC = available non-structural carbohydrate; AVN = available non-structural N.)

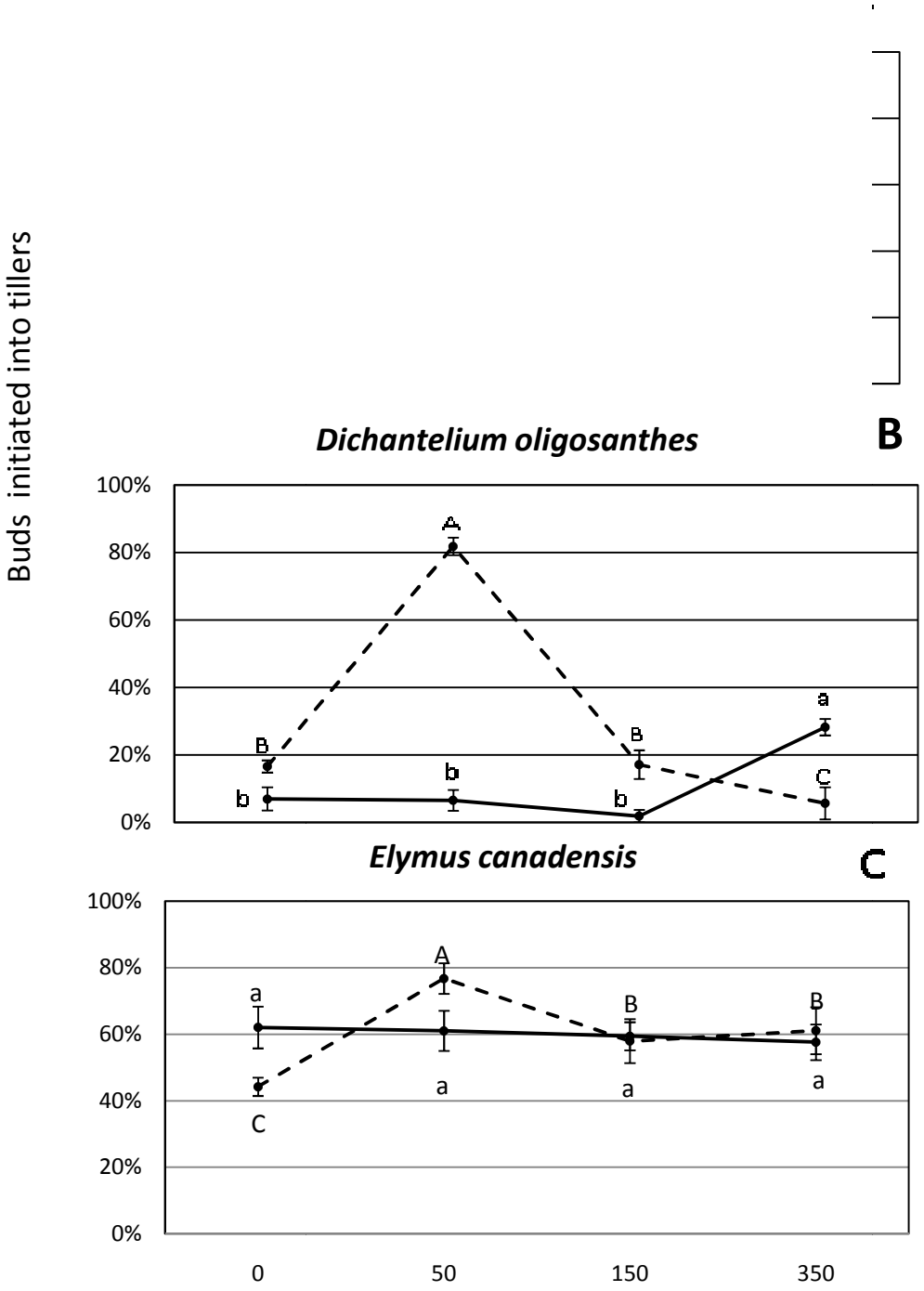

Figure 1-2. Percentage of buds (±1 SE, n=6) that initiated into tillers from three C_3 cool-season grass species when treated with either water as a control (0) or one of three concentrations of NH_4NO_3 amendment, Low (50 mg/L), Medium (150 mg/L), or High (350 mg/L). Solid lines designate dark treatments and dashed lines designate light treatments. Mean percentages with the same letter are not significantly different at the 0.05 level. Lower-case letters designate statistically significant differences in dark treatments; capital letters designate statistically significant differences in light treatments.

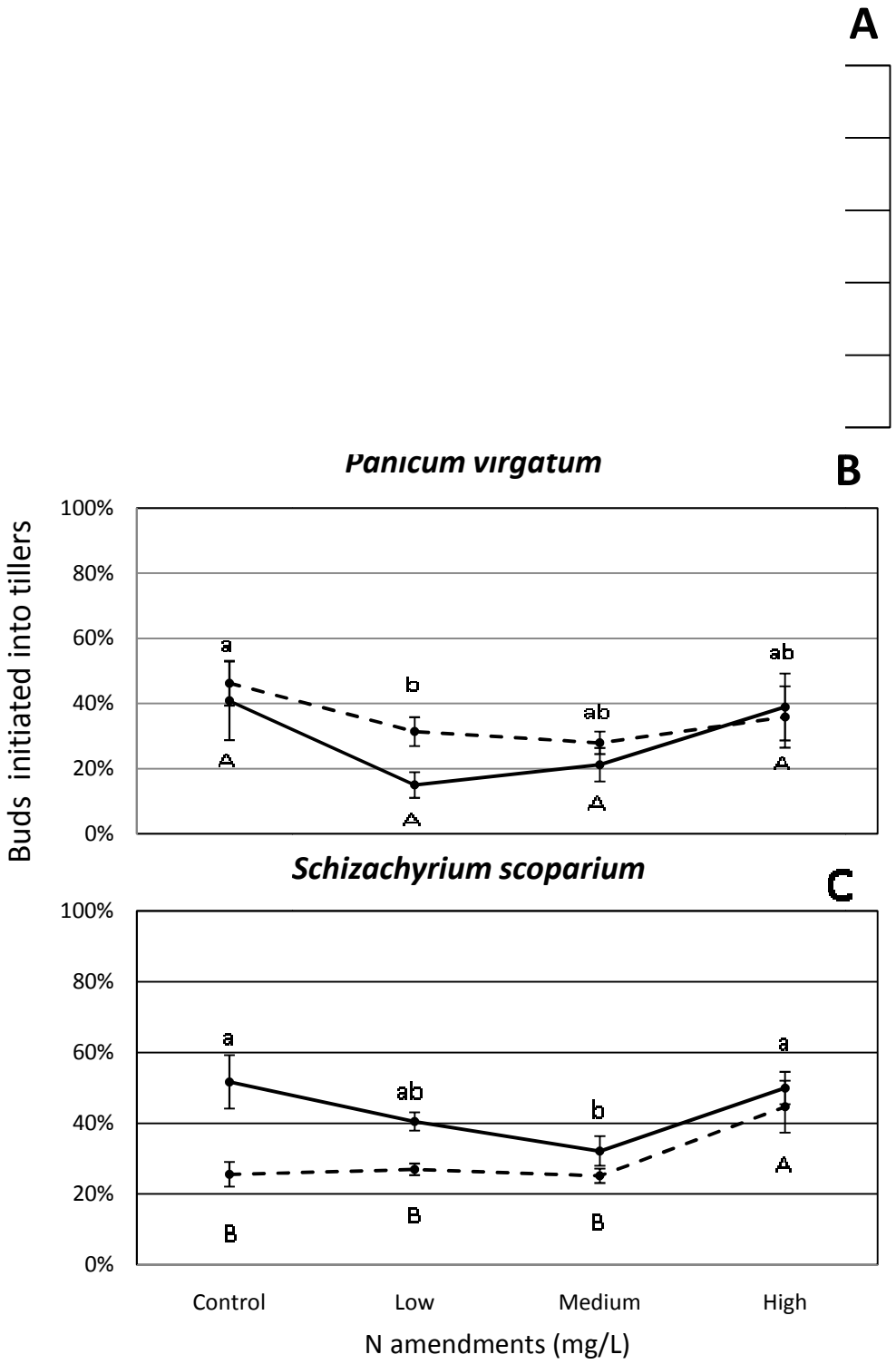

Figure 1-3 Percentage of buds (±1 SE, n=6) that initiated into tillers from three C_4 warm-season grass species when treated with either water as a control (0) or one of three concentrations of NH_4NO_3 amendment, Low (50 mg/L), Medium (150 mg/L), or High (350 mg/L). Solid lines designate dark treatments and dashed lines designate light treatments. Mean percentages with the same letter are not significantly different at the 0.05 level. Lower-case letters designate statistically significant differences in dark treatments; capital letters designate statistically significant differences in light treatments.

CHAPTER II

Effects of Light Spectral Quality and Availability on Bud Banks

ABSTRACT

Three species of C_3 grasses and three species of C_4 grasses were studied to test a proposed theory (Tomlinson and O'Connor 2004), to determine the role that light spectral quality (R:FR) and light quantity have on belowground bud outgrowth. I hypothesized that (1) R:FR will be an important cue in the regulation of bud bank dynamics, (2) reductions in light quantity due to litter accumulation will significantly reduce bud outgrowth, and (3) the response to the two factors will not be consistent across the two functional groups. Environmental growth chambers were used to conduct all study treatments. Plants were placed in chambers under one of three light treatments: light, reduced R:FR, or a full dark (control). To assess the effects of light availability under natural conditions, litter was used to reduce light availability in the C_4 species. Light spectral quality and quantity elicited species-specific responses in both of the functional groups. A suppression in bud outgrowth in response to R:FR reductions were observed in four of the six species. Alterations to light quantity due to litter accumulation did not reduce bud outgrowth, but a reduction in R:FR did, suggesting that light spectral quality

is a more important regulator of bud dormancy and outgrowth than light intensity. Surprisingly, the response to light spectral quality and quantity were consistent across both functional groups. As we continue to seek answers to enhance rangeland quality, assessing how environmental cues are interacting may be a stronger predictor of how rangelands will respond to current and future global changes, than assessing the cues individually.

INTRODUCTION

The grasslands in the Great Plains region of North America occupy more than 1.5 million km^2 of land area. They are considered complex, yet underappreciated ecosystems (Knapp and Seastedt 1998), characterized by specific moisture and precipitation requirements. Many of the grass species in this ecosystem reproduce primarily by belowground vegetative perennating organs and therefore populations are maintained primarily through the maintenance of their belowground population of dormant meristems or "bud banks" (Harper 1977). The key role that bud banks play in the recruitment of aboveground tillers in these systems have been found to be of much greater importance in plant population persistence and dynamics than the seed rain or seed banks (Glenn-Lewin et al. 1990, Pyke 1990, Hartnett & Keeler 1995, Hartnett & Fay 1998, Benson et al. 2004, Benson & Harnett 2006). Dalgleish and Hartnett (2006) suggest that grassland ecosystems with relatively large reserves of belowground bud banks may be the most resilient to global change phenomena. As changing abiotic environmental conditions continue occurring on a global level (e.g. regional N enrichment and alterations in precipitation and temperature), the important role that these factors have in structuring community composition within grassland and savanna

ecosystems may have a larger impact than previously thought (Dalgleish and Hartnett 2006).

In mesic tallgrass prairie ecosystems, nitrogen, water, and light are three resources that limit plant productivity, with nitrogen being the most limiting factor in these systems (Owensby et al. 1970, Seastedt and Knapp 1993, Knapp et al. 1998b). Consequently, population dynamics of prairie plants, as well as vegetative growth and production are affected by this limitation. Because variation in aboveground net primary production (ANPP) in tallgrass prairie ecosystems can be attributed to differences in tiller density, rather than tiller size (Hartnett and Fay 1998), resources such as these may be critical in regulating bud dormancy and tillering. Previous research conducted in the tallgrass prairie has indicated increased annual precipitation to be positively correlated with increased bud bank densities across a number of grassland sites (Dalgleish and Hartnett 2006), and nitrogen addition had positive effects on bud outgrowth and tiller emergence and genet growth rates in some grassland species (Dalgleish et al. 2008).

Light is a critical resource for plants, influencing photosynthetic rates, growth, and reproduction. Light spectral quality, in particular red and far red light, and light intensity have all been documented as regulators of various plant development and morphological changes (Agren 1985, Casal & Smith 1989, Chory et al. 1994, Bonser and Aarsssen 2003, Sherry et al. 2008). The red:far red ratio (R:FR) has been found to serve as a warning signal for competition for light and plants have been found to respond to this signal in a number of ways, including elongating stems, reducing branch number, increasing leaf area and increasing aboveground tillers (Tucker 1975, Smith 1982, Deregibus et al. 1983, Ballare et al. 1994). Studies conducted on grasses have primarily

focused on the aboveground responses of plants to light quantity and light spectral quality, with most research focusing on the response of tillering, ramet height, and sheath size (Casal et.al. 1989, Monaco and Briske 2000). Less however, is known about the influence of these factors on bud production, dormancy, activation, and tiller initiation in grasses in these systems.

Based on synthesis of previous studies assessing the mechanisms stimulating dormant bud outgrowth in grasses, Tomlinson & O'Connor (2004) proposed an integrated model, listing three specific mechanisms interacting to trigger bud outgrowth. Their aim was to link key mechanisms responsible for lateral bud outgrowth. They key mechanisms proposed in their model include, resource (nitrogen) availability, apical dominance, and photosensitivity to the (R:FR) (Briske and Derner 1998). Their goal was to achieve a better understanding of what drives bud/tiller dynamics. They hypothesized that soil available N and light spectral quality (R:FR ratio) are the two key environmental factors regulating bud dormancy and tiller recruitment from bud banks. This suggests that environmental changes that alter light spectral quality and N availability (e.g. land use change and regional N enrichment), may have the greatest impact on the productivity and dynamics of grassland vegetation.

Scientists currently have an abundance of evidence on globally occurring changes in the climate, nutrient enrichment, and exotic species invasion, due to increases in human populations, deforestation, fossil fuel consumption, and intensification of agriculture (Matson et al. 1997, Vitousek et al. 1997, Sax and Gaines 2003, Trenberth et al. 2007). Previous studies on the response of C_3 and C_4grass species to changing environmental cues have observed shifts in physiological groups with C_4 grass species

increasing with warming (Wan et al. 2005) and C_3 species increasing with long-term increases in water availability (Knapp et al. 2001) in grassland ecosystems. As the evidence of ongoing climate change continues to mount, having a sound understanding that environmental cues have on the regulation of bud development, dormancy, and dynamics is essential to accurately predicting rangeland responses to future changes in abiotic environmental conditions.

Changing environmental phenomena may also affect light quantity. In grasslands, detritus is a key regulator of NPP by altering the light environment at the soil surface, as well as affecting energy flow and water and nitrogen movement (Knapp and Seastedt 1986). In productive rangelands, the quantity of photosynthetically active radiation (PAR) at soil level may be reduced by 58% relative to sites without detritus (Knapp 1984). Changes in land use practices, such as decreases in fire frequency, increases litter accumulation may potentially have significant effects on bud activation and tiller initiation, as this will alter the quantity of light that reaches the soil surface.

The key objective of this study is to test the Tomlinson and O'Connor (2004) model predictions regarding environmental cues regulating bud dynamics. In Chapter 1, I addressed their model predictions regarding the role of nitrogen as a cue that promotes bud outgrowth and tiller initiation in tallgrass species and I will expand on their model predictions by specifically looking at light spectral quality and light availability in this chapter. The general applicability of this model to different rangeland grasses will be assessed by examining these factors that potentially influence bud dormancy and activity in four rhizomatous and two caespitose grass species from a mesic North American rangeland.

The purpose of my study was to test three hypotheses based on the Tomlinson and O'Connor's (2004) model predictions:

H_1. R:FR will be an important cue in the regulation of bud bank dynamics.

H_2. Reductions in light availability due to litter accumulation will significantly reduce bud outgrowth.

H_3. The responses to the two environmental cues will differ between the two functional groups (e.g. C_3 species and C_4 species).

This research will empirically test the model predictions laid out by Tomlinson and O'Connor (2004) (see also chapter 1). This information will be used to provide a better understanding of the effects that changes in the quantity and quality of light may have on the mechanisms driving patterns of variability in productivity in these systems, in hopes of predicting responses in the future.

MATERIALS AND METHODS

Collection Site:

The collection of plant material was conducted at Konza Prairie Biological Station (KPBS), a tallgrass prairie research site, located in the Flint Hills of northeastern Kansas (39°05' N, 96°35' W). KPBS is a 3487-ha Long Term Ecological Research site, owned by The Nature Conservancy and Kansas State University. The preserve is managed for ecological research by the Kansas State University, Division of Biology. KPBS is a temperate, mesic grassland, characterized by a temperature-moisture regime under which a variety of plant types can coexist, with over 550 higher plant species recorded (Knapp and Seastedt 1998,Towne 2002). Prairie vegetation is dominated by

warm-season C_4 perennial grasses, with both rhizomatous and caespitose growth forms, with numerous species of cool-season graminoids, composites, legumes and other forbs present as well. KPBS is divided into 64 watershed units, each containing distinct topographical gradients, including an upland, slope, and lowland areas. Fire and grazing regimes are also assigned to each watershed. Fire regimes occur in 1-, 2-, 4-, 10-, and 20-year frequencies and grazing regimes include grazed or ungrazed by native herbivores (e.g. bison) and cattle. Rhizomes for this study were collected from annually burned ungrazed watersheds.

Selection of Species:

Photosynthetic pathways of C_3 and C_4 species play a critical role in flowering phenology, growth period, and ultimately geographic distribution. C_4 or warm-season grass species focus their growing season throughout the summer months, with flowering typically occurring in the late summer months. During this period of new tiller growth and maturation, new buds are forming, joining older buds, to form the overwintering dormant bud bank, some of which will subsequently initiate and tiller the following year during the growing season. Alternatively, C_3 or cool-season grass species have a growing season that begins in early spring, with flowing occurring from April to June. Production of buds occurs during this period as well, and continues throughout their growing season. Studies conducted on the C_3 species *D. oligosanthes*, and the C_4 species *A. gerardii*, showed that while both species became dormant over the winter season, *A. gerardii* used buds to overwinter, while *D. oligosanthes* used juvenile tillers to survive the winter dormancy. A second difference in the bud bank dynamics between the two groups is that

D. oligosanthes has an additional dormancy period in the summer due to high temperatures, where is used bud to survive the dormancy (Ott 2009).

Andropogon gerardii (big bluestem) and *Panicum virgatum* (switchgrass) are dominant C_4 perennial grass species found on KPBS and reproduce vegetatively via belowground rhizomes. *Schizachyrium scoparium* (little bluestem) is also a dominant C_4 perennial grass found on KPBS but this species is characterized by a caespitose growth form (Towne 2002). *Koeleria macrantha* (prairie junegrass) and *Dichanthelium oligosanthes* (Scribner's panic grass), are two subdominant C_3 perennial bunchgrasses that are commonly found on the upland areas of KBPS. *Elymus canadensis* (Canada wildrye), also a C_3 perennial grass, is a subdominant species that has a rhizomatous growth form and is found across all prairie sites (Towne 2002).

The belowground perennating organs (e.g. crowns and rhizomes) of established C_4 grasses (*Andropogon gerardii*, *Schizachyrium scoparium* and *Panicum virgatum*) with their dormant buds and C_3 grasses (*Koeleria macrantha*, *Elymus canadensis*, and *Dichanthelium oligosanthes*) with their dormant buds were collected on watersheds N1A, K1B, and 1D on KPBS. C_4 materials were collected in February, when their buds were dormant, while the C_3 rhizomes were collected in July, corresponding to their summer bud dormancy (Ott 2009).

Belowground Sampling:

Plant materials were harvested to a depth of 10 cm and all interconnected and belowground structures (buds, rhizomes, roots, etc) were stored in large plastic containers and transported to Oklahoma State University. The samples were washed free of soil, sorted by species, and roots trimmed to 3 cm. Rhizomes were then placed into flats of

moistened Medium A-2 grade vermiculite and were refrigerated at 2-4°C in complete darkness until experimental set-up.

Experiment Set-up:

Experiments were conducted in Controlled Environmental Chambers, (Conviron-PGW 36, interior dimension: 98"Wx54"Dx93"H, growth area: 36ft^2, and growth capacity: 240 ft^2), housed within the Controlled Environmental Research Lab (CERL) on the campus of Oklahoma State University in Stillwater, OK. Replicate studies were conducted on all six species of C_4 and C_3 grasses in the spring and fall of 2009. Perennating organs of similar size of each individual species were transplanted into square plastic pots (9 cm length x 8 cm depth) containing vermiculite. Each perennating organ was placed within 1 cm of the vermiculite surface and randomly arranged in growth chambers. Because we observed green tillers during the dormant season collection on both *D. oligosanthes* and *K. macrantha*, prior to placing these species into pots, live tillers were counted and recorded.

To assess the effects of bud outgrowth under photoperiods and temperature regimes similar to actual conditions in the tallgrass prairie during the growing season for each functional group, chambers were set under a photoperiod of 14 hours at 30/20°C day/night temperature for the C_4 (warm-season) species and 20/12°C day/night temperatures for the C_3 (cool-season) species. A full dark treatment was included as a control. Each species had six replicates subjected to each of the nitrogen treatments, for a total of 24 pots/species in the light treatment and 24 pots/species in the dark control treatment.

Plants were monitored and irrigated every 2-3 day, and the number of buds released from dormancy and emerging as juvenile tillers were counted and recorded daily for 3 weeks or until no new tiller initiations were observed. The belowground plant components were then washed free of vermiculite and number of live buds and live tillers (>2cm) present were counted using a dissecting microscope. The number of live residual buds + the number of live newly emerging tillers provides the starting number of buds on the belowground structures at experimental set up.

Light Intensity:

C_3 species- Light intensity treatments were established using (1) a full light treatment (250 µmol/m^2/s) and (2) a no light (dark) control (Bonser & Aarssen 2003).

C_4 species- The same light treatments as described for my C_3 species were used for C_4 species. In addition, to assess the effects of light availability under more natural conditions, litter was used to reduce light availability. Litter was collected in summer 2009 from an infrequently burned watershed on KBPS. The litter was sterilized at 90° C to avoid pathogen contamination, and added to individual pots at depths of 0 (no litter), 5, or 10 cm to examine the effects of light intensity on bud initiation. For each litter treatment, PAR was measured at the soil surface using a Decagon Sunfleck Ceptometer.

Light Spectral Quality:

To test the effects of light availability on bud bank initiation and tillering, a layer of green plastic film (Filter HT*121, Lee Filters, Andover, UK) was positioned between the light source and the pot surface, producing a R:FR ratio of 0.2 (Bonser & Aarssen 2003). A double layer of clear plastic film was positioned in the same fashion over

control pots, as a means to control for any unintended effects of the plastic film on the microclimate. Light spectral quality in each treatment was monitored by measuring incident radiation at wavelengths from 300 nm to 800 nm using a spectroradiometer. The R:FR ratio was then calculated from the incident radiation at 660 nm (red) and 730 nm (far red). Total incident radiation (PAR) was measured in each chamber. Using Beer's law, total irradiance was adjusted by varying distance between light source and canopy to insure identical total irradiance at soil/tiller level and no confounding effects of filters on total incident light intensity. An additional set of rhizomes was placed in 24 hours dark, as a control. A control treatment was set up, where rhizomes were placed in 24 hours of dark.

Statistical analysis:

The following equation was used to determine the proportion of buds that broke dormancy and grew out into juvenile tillers in each of the treatments:

$$\frac{T_e}{T_e+(B_d-T_i)} = \text{proportion of buds initiating into tillers}$$

where T_e=number of emerging tillers (>2cm) and B_d=number of remaining dormant (inactive) buds recorded at the end of the experiment for each replicate, and T_i = number of juvenile tillers present at the beginning of the experiment. Proportional data of C_4 species were logit transformed prior to analysis to reduce heterogeneity of variances. Untransformed data are presented on figures. For each species, the effects of light quality and light availability on the proportion of bud producing tillers were analyzed via two-way ANOVA using SAS version 9.2 software (SAS Institute Inc, Cary, NC, USA), and the significant levels of differences are reported for $P \leq 0.05$.

RESULTS

Response of C_3 grasses to light spectral quality treatments:

All three of the C_3 grass species responded differently to the three light treatments (Figure 2-1, A-C). Light increased the activation of meristems in *K. macrantha* by 10% compared to dark conditions and the reduced R:FR ($P<0.05$). Reducing the R:FR also significantly suppressed bud outgrowth in *E. canadensis*, by 15% compared to the light treatment and 10% compared to the dark treatments. Plants in full light and total dark treatments however, responded similarly in their initiation of buds. The third C_3 species, *D. oligosanthes*, exhibited no difference in tiller initiation across all treatments (Figure 2-1, A-C).

Response of C_4 grasses to light spectral quality treatments:

Two of the C_4 species responded similarly to the four light treatments. *Panicum virgatum* and *S. scoparium* tiller initiation was suppressed when exposed to reduced R:FR by nearly 10% compared to both light and dark treatments for each species, while neither full light nor reduced light intensity due to litter treatments affected tiller recruitment compared to the control (dark treatments) (Figure 2-2, B, C). Changes in light spectral quality did not affect *A. gerardii* tiller initiation (Figure 2-2, A).

DISCUSSION

Light spectral quality and quantity elicited species specific responses in both of these two functional groups. Sensitivity to a reduction in the R:FR was observed in four of the six grass species, while no detectable differences were observed between light and dark treatment in any of the six species, therefore the first hypothesis can be accepted. The suppressed tiller initiation in response to reductions in R:FR are consistent with Tomlinson and O'Connor's (2004) predictions, based on previous studies on the R:FR

effects on tillering. They determined that R:FR was a flexible species trait, that the effects were inconsistent among species, and that even within a localized population, the same species can display both sensitivity and insensitivity to R:FR depending on location. Although these predictions were based on studies of C_3 grass species, the same species specific responses were observed in the C_4 grass species as well.

My second hypothesis, that alterations in light quantity due to litter accumulation will reduce bud outgrowth, was not supported by my data. I observed no significant differences between litter, light, and dark treatments in any of the C_4 grass species. I did, however observe a significant difference between reduced R:FR light, with R:FR light suppressing bud outgrowth in two of the three C_4 species. It is also important that the affects of light quality were not consistent across all species, but rather appears to be species-specific. This research is in contrast to previous studies that found that tillering is reduced in undisturbed prairie sites relative to burned prairie sites, as these authors concluded the effects were due to higher light availability (Weaver and Rowland 1952, Hulbert 1969).

Because I observed generally consistent results across all functional groups for my light and dark treatments, my third hypothesis will be rejected. Similar reactions to R:FR in two of the C_4 species and two of the C_3 species, further document that species respond similarly to one another, despite differences in their physiology. As mentioned above, the effect of light spectral quality appears to play a role in the suppression of bud outgrowth, but light quantity did not affect the number of buds that initiated into tillers. The factors that remained consistent throughout the growth chamber study were water quantity, temperature, and humidity. All of these factors could be playing a role, by

working alone or together, to make conditions ideal for bud initiation in these species, despite the amount of available light. Low humidity has been shown to inhibit vegetative outgrowth in C_3 plant species, but this could be counteracted with the addition of water to the rhizome or exposure of the rhizome to light (McIntyre 1981). Water availability also plays a role in bud bank densities and bud outgrowth, with increases in water availability corresponding to increases in bud bank densities (Dalgleish and Hartnett 2006).

This is not to say that these are the only factors that may be playing a role or multiple roles in conjunction with light spectral quality. As mentioned above, I found no significant differences between light and dark treatments in any of the species in my study. Tomlinson and O'Connor (2004) proposed that the ratio of auxin and cytokinins play a key role in tillering in grasses. Both of these hormones play important, but opposing roles in bud dormancy and outgrowth. The hormone auxin has been shown to regulate apical dominance, the inhibition of lateral (axillary) bud outgrowth, while a second group of hormones, cytokinins promote cell division, lateral bud outgrowth, and the mobilization of plant nutrients (Taiz and Zeiger 2006). Recent studies suggest that phytochrome sensitivity to R:FR may actually manipulate auxin rates, resulting in an inhibition of bud outgrowth (Cline 1994, Kraepiel and Miginiac 1997, Tomlinson and O'Connor 2004). This may be a reasonable explanation for the suppression of bud outgrowth in response the reduction of R:FR light spectral quality observed in my study.

The role that light quality and quantity play in bud outgrowth and tillering in native grass species could potentially affect grassland ecosystem dynamics in the future, as our environment continues to change. As the human population continues to increase, anthropogenic impacts on land use that cause changes in litter depth or promote woody

encroachment or successful invasion (e.g. manipulation of fire regimes, intensive grazing) have the potential to alter the light spectral quality and light quantity in grassland ecosystems (Vitousek et al. 1997, Van Drecht et al. 2005). As we continue to seek answers to enhance rangeland quality, we must take a close look at how these environmental cues are interacting. These interactions may be important in determining future patterns of species composition and bud bank dynamics in tallgrass prairie ecosystems in response to current and future global changes

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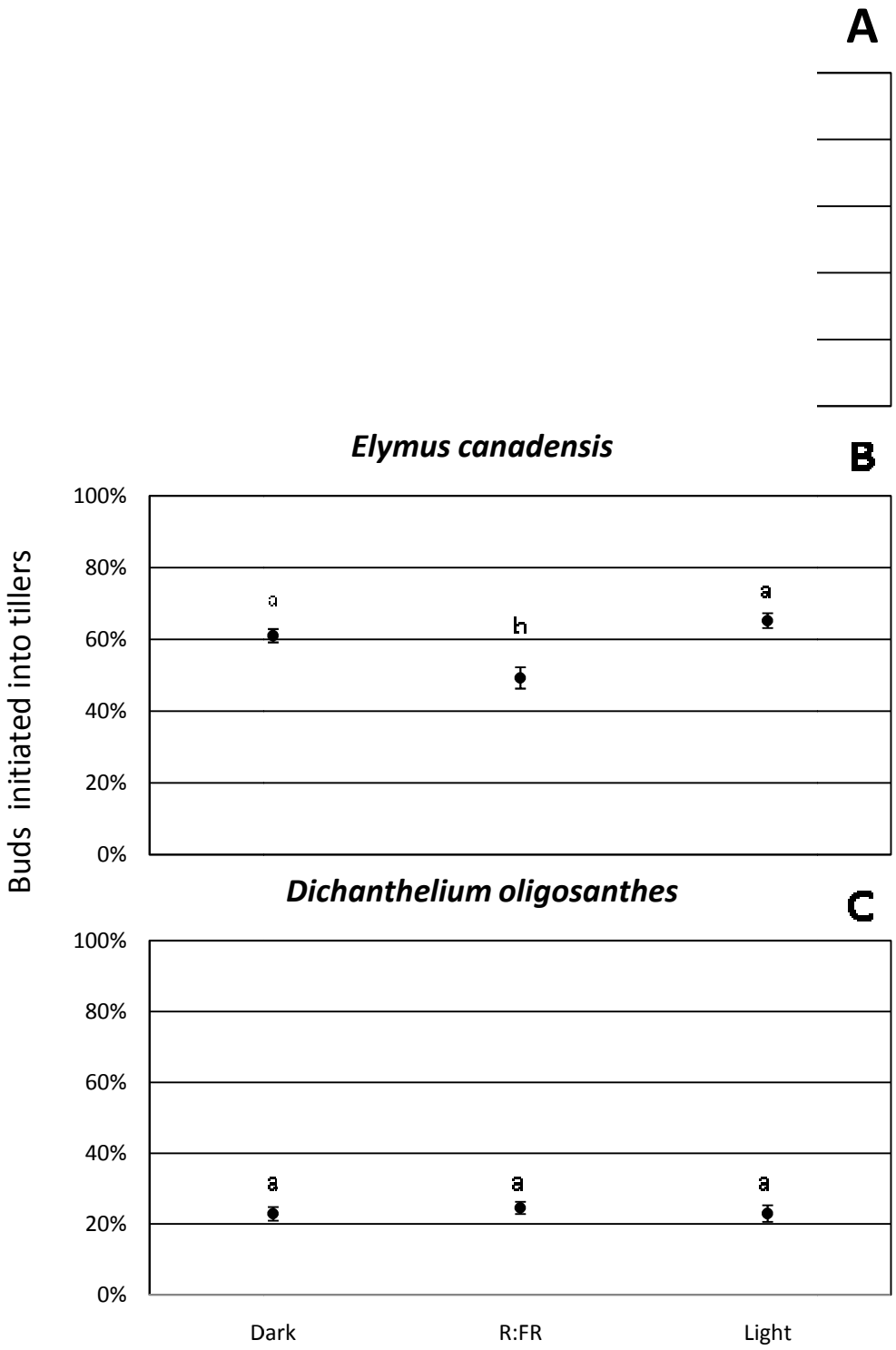

Figure 2-1. Percentage of buds (±1 SE, n=12) that initiated into tillers when exposed to one of four light treatments from three C_3 cool-season grass species. Light treatments are: No light (dark), 30% reduction of red-far red light, or full light. Mean percentages with the same letter are not significantly different at the 0.05 level.

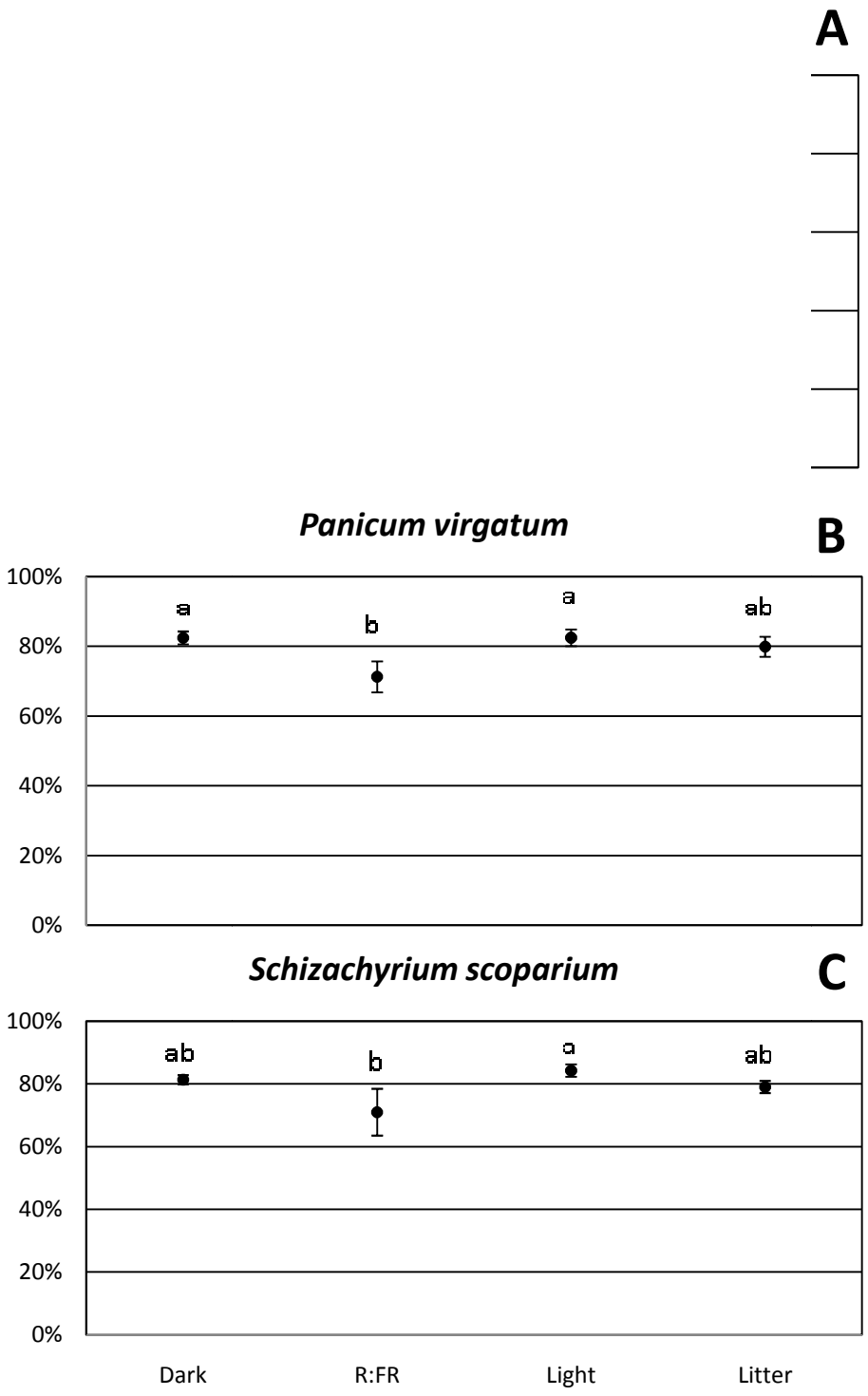

Figure 2-2. Percentage of buds (±1 SE, n=12) that initiated into tillers when exposed to one of four light treatments from three C_4 warm-season grass species. Light treatments are: No light (dark), 30% reduction of red-far red light, full light, or reduced light due to litter cover. Mean percentages with the same letter are not significantly different at the 0.05 level.

VITA

Melinda Marie Williamson

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Master of Science

Thesis: ASSESSING THE EFFECTS OF ENVIRONMENTAL CUES ON BUD ACTIVATION AND TILLER INITIATION IN RANGELANDS

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Scope and Method of Study: Perennial grass populations in tallgrass prairie ecosystems are maintained primarily through vegetative outgrowth from their belowground bud banks. Having a sound understanding of the proximal abiotic environmental cues that regulate bud development, dormancy, and outgrowth is essential to accurately predict rangeland responses to changing abiotic environmental conditions. I tested the Tomlinson and O'Connor theory on the interactions of nitrogen, light spectral quality and light availability and the roles they have in bud outgrowth and tiller initiation in six perennial grass species in two functional groups (C_3 and C_4 photosynthetic pathways). Studies were conducted in controlled environmental chambers. To test the effects of nitrogen on bud outgrowth, plant were supplemented with one of three NH_4NO_3 amendments (low, medium, or high) or water (control). To additionally test for an interaction between nitrogen and light availability, plants were exposed to either a light treatment or a dark (control) treatment. In a second experiment, the effects of light spectral quality and light availability were assessed by exposing plants to either a full light, reduced R:FR light, reduced light by litter, or full dark (control). Assessments of the proportion of buds that broke dormancy and grew out into juvenile tillers in each treatment were compared using analysis of variance.

Findings and Conclusions: The effects of varying nitrogen levels and light availability were different between functional groups. Strong interactions between nitrogen and light influenced bud outgrowth in the C_3 species, but not in the C_4 species. In the second study, functional groups responded similarly in response to light quality and availability. A suppression in bud outgrowth in response to R:FR reductions were observed in four of the six species while alterations to light quantity due to litter accumulation did not reduce bud outgrowth in any of the species. As we continue to seek answers to enhance rangeland quality, assessing how environmental cues are interacting may be a stronger predictor of how rangelands will respond to current and future global changes, than assessing the cues individually.

ADVISER'S APPROVAL: Dr. Gail Wilson